CLAIMS

Claim 1

A method to characterize a T-cell response of a final population of T-lymphocytes resulting from the co-incubation of an initial population of T lymphocytes with a composition of antigen-presenting cells (APCs), said method comprising the following steps:

1. the simultaneous measure on a single cell basis of at least two parameters:
   i. the first parameter being necessarily proliferation of T lymphocytes,
   ii. the second parameter being necessarily chosen among the group consisting of presence of a T cell antigen receptor on the surface of T lymphocytes and presence of at least one biological molecule produced by T lymphocytes,

and the attribution of a positive or a negative value to each of these parameters,

2. the classification of the final T-lymphocytes population into 2^n different subsets of T lymphocytes, n being the number of parameters used for the measure, each subset being characterized by a positive or a negative value respectively to each parameter, and the determination of the proportion of T lymphocytes present in each subset with respect to the number of T lymphocytes in the final population, with said proportion being characteristic of the T-cell response.

Claim 2

The method according to claim 1, wherein the step (1) comprises the measure of an additional parameter being the presence or not of at least one surface determinant marker on T lymphocytes, different from T cell antigen receptors.

Claim 3

The method according to claim 1 or 2, wherein antigen-presenting cells are loaded with at least one antigen or fragment of antigen.

Claim 4

The method according to claim 3, wherein the T cell antigen receptor on the surface of T lymphocytes is specific or not for an antigen or fragment of antigen loaded on said APCs.

Claim 5

The method according to the claim 4, wherein the T cell antigen receptor on the surface of T lymphocytes is specific for an antigen or of a fragment of antigen loaded on said APCs.

Claim 6

The method according to any one of claims 1 to 5, comprising a third step of determination, from the proportion of T lymphocytes in each of the different subset present in the final population, of the proportion of T lymphocytes in each corresponding subset present in the initial population with respect to the number of T lymphocytes in the initial population.

Claim 7

The method according to claim 6, wherein the determination of the proportion of T lymphocytes present in the initial population of T-lymphocytes loaded with a fluorescent probe allowing the measure of proliferation is made according to following steps:

(i) marking n minus 1 parameters, the parameter corresponding to the proliferation being previously marked, with fluorescent probes specific for each of the n minus 1 parameters,

(ii) gating T-lymphocytes in the final population of T lymphocytes according to the fluorescence of the n minus 1 chosen parameters, the measure of proliferation being excluded at this step, the value of which define lymphocytes subsets of interest,

(iii) building a fluorescent curve by recording the fluorescence intensity of the probe used to measure proliferation of the T-lymphocytes gated at step (ii),

(iv) possibly building a fluorescent curve by recording the fluorescence intensity of the probe used to measure proliferation from either:

(iva) T-lymphocytes present in a lymphocytes subset defined by gating lymphocytes in the final population of T lymphocytes according to the absence of fluorescence of the n minus 1 chosen parameters or,

(ivb) T-lymphocytes present in a sample of T-lymphocytes of the initial population not co-incubated with APCs,

(v) determining intensity of fluorescence of non-proliferating lymphocytes by analyzing the distribution of fluorescence of the fluorescent curve built at step (iii), or possibly at step (iv), the non-proliferating lymphocytes corresponding to the maximal value of fluorescence,

(vi) deriving, from the fluorescence curve recorded at step (iii), Gaussian curves centered on successive half intensity values derived from the maximal intensity of fluorescence determined from non-proliferating T-lymphocytes at step (iii) or at step (iv), to obtain A_k which is the proportion of cells in division k at the time of the measure of the proliferation,

(vii) determining the proportion of T-lymphocytes (PF=precursor frequency) in the initial population that have proliferated in order to give the proportion of T lymphocytes present in the selected subset (step ii) using the formula:

$$PF = \left(\sum_{k=2}^{k=n} A_k/2^k \right) \Big/ \left(\sum_{k=0}^{k=n} A_k/2^k \right)$$

wherein PF is precursor frequency in the initial population, A_k is the proportion of cells in division k at the time of the measure of the proliferation, k=0 for initial population of T lymphocytes, and cells having undergone 2 to n divisions having been classified as proliferating T lymphocytes,

(viii) determining the percentage of non-proliferating T-lymphocytes from the percentage of T-lymphocytes that have not proliferated and that are present in the final population of T-lymphocytes and half the percentage of T-lymphocytes that had undergone only one cell division,

(ix) applying the percent of non-proliferating T-lymphocytes to the number of gated T-lymphocytes in the data file to give the absolute number of T-lymphocytes in the corresponding subset before culture that will not proliferate according to the formula,

$$\text{number}_{\text{non-proliferating cells in the initial population}} = [(\text{proportion}_{\text{cells that have not proliferated and that are present in the sample at the end of the experiment}}) + (0.5 * \text{proportion}_{\text{cells that have divided only once and that are present in the sample at the end of the experiment}})] * [\text{number}_{\text{gated cells in data file}}],$$

(x) determining the absolute number of T-lymphocytes in the corresponding subset destined to divide by knowing the number of T-lymphocytes that was not destined to divide and the number of precursor cells of proliferating T-lymphocytes according to the formula,

$$\text{number}_{\text{proliferating cells in the initial population}} = [(PF_{\text{proliferating cells}}) * (\text{number}_{\text{non-proliferating cells in the initial population}})] / [1 - PF_{\text{proliferating cells}}],$$

(xi) reiterating step (i) to step (viii) to each T lymphocytes subsets determined according to the n parameters used for the measure,

(xii) summation of number of cells in the 2^n subsets in order to express the number of T-lymphocytes present in the initial population of T-lymphocytes as a percentage of the total initial population before co-incubation.

Claim 8

The method according to any one of claims 1 to 7 wherein APCs are monocyte-derived antigen presenting cells.

Claim 9

The method according to any one of claims 1 to 8, wherein APCs are immature, maturing or mature dendritic cells, monocytes or macrophages.

Claim 10

The method according to any one of claims 3 to 9, wherein APCs are loaded with at least one antigen or a fragment of antigen which is an antigen of tumoral or infectious origin.

Claim 11

The method according to claim 10, wherein said antigen is comprised in the group consisting of: EBV, CMV, HBV, p53, tetanus toxin, Melan-A/MART-1, MAGE-3, MAGE-2, PSA, PSMA, PAP, HSP70, CEA, Ep-CAM, MUC1, MUC2, HER2/neu, M1 protein from the influenza virus, or peptides derived from these proteinic antigens.

Claim 12

The method according to any one of claims 1 to 11, wherein co-incubation of APCs and T lymphocytes lasts for a time sufficient to allow at least 1 division of T lymphocytes, preferably 5 divisions.

Claim 13

The method according to claim 12 wherein co-incubation lasts from 1 to 10 days, and more preferably 4 to 10 days.

Claim 14

The method according to any one of claims 1 to 13, wherein co-incubation comprises a step of adding a T lymphocyte stimulating agent at the end of the co-incubation period.

Claim 15

The method according to any one of claims 1 to 14, wherein proliferation of T lymphocytes is measured by using a probe loaded into T lymphocytes before or concomitantly to the step of co-incubation, said probe being substantially equally distributed between dividing T-lymphocytes during cell division of T lymphocytes of the initial population.

Claim 16

The method according to claim 15, wherein the probe loaded before the co-incubation step is fluorescent and distributed in the cell membrane or inside the cytosol of T-lymphocytes.

Claim 17

The method according to claim 15, wherein the probe loaded into the T-lymphocytes concomitantly to the co-incubation step is a base analog that integrates into the DNA, such as BrdU.

Claim 18

The method according to any one of claims 1 to 17, wherein the presence of a specific T cell antigen receptor on the surface of T lymphocytes is detected by using a detectable molecule having the ability to specifically bind to said T cell antigen receptor, such as a fluorescent tetramer.

Claim 19

The method according to any one of claims 1 to 18, wherein the T cell antigen receptors on the surface of T lymphocytes are specific for an antigen from tumor or infectious origin.

Claim 20

The method according to claim 19, wherein said antigen is chosen among the group consisting of: EBV, CMV, HBV, M1 protein from the influenza virus, p53, tetanus toxin, Melan-A MART-1, MAGE-3, MAGE-2, PSA, PSMA, PAP, HSP70, CEA, Ep-CAM, MUC1, MUC2, HER2/neu, or peptides derived from these proteinic antigens.

Claim 21

The method according to any one of claims 1 to 20, wherein the biological molecule whose presence is detected in final population of T lymphocytes, is a cytokine or a chemokine or an enzyme.

Claim 22

The method according to claim 21, wherein the cytokine is chosen among the group consisting of: IFN-γ, IL-2, IL-4, IL-5, IL-10.

Claim 23

The method according to claim 21, wherein the enzyme chosen among the group consisting of perforine and granzyme.

Claim 24

The method according to claim 21, wherein the chemokine is chosen among the group consisting of a ligand for CCR5 and a ligand for CCR7.

Claim 25

The method according to any one of claims 2 to 24, wherein the surface determinant marker of T-lymphocytes is chosen among the group consisting of CD4, CD8, CD28, CD69, CTLA-4, CD45-RA, CD45-RO, CD62-L.

Claim 26

The method according to any one of claims 1 to 25, wherein the proportion of T lymphocytes in the different subsets of T lymphocytes in the final population and the proportion of T lymphocytes in the different corresponding subsets of T lymphocytes in the initial population are used to determine a proliferation index (PI) for each subset of T lymphocytes using the formula:

$$PI = \left(\sum_{k=0}^{k=n} A_k \right) \Big/ \left(\sum_{k=0}^{k=n} A_k / 2^k \right)$$

wherein A_k is the proportion of cells in division k

Claim 27

Use of a method according to any one of claims 1 to 26, as a potency assay for a composition of APCs.

Claim 28

The use of a method according to claim 27, wherein the APCs ability to activate T lymphocytes is characterized by the determination of the proliferation index and/or by the proportion of T lymphocytes precursors present in the initial population.

Claim 29

The use of a method according to claim 28, wherein APCs are characterized by their ability to induce proliferation of T lymphocytes, positive at least for the proliferation parameter, resulting in a proliferation index of at least 2, more preferably of at least 5, more preferably of at least 10, more preferably of at least 15, more preferably of at least 20, more preferably of at least 30, more preferably of at least 50.

Claim 30

The use of a method according to claim 29, wherein APCs are characterized by their ability to induce a proliferation of T lymphocytes, positive at least for the proliferation parameter, resulting in a proliferation index ranging between 2 and 200, more particularly from 15 to 70, more particularly from 20 to 60, more particularly from 30 to 40 and more particularly from 20 to 200.

Claim 31

Use of a method according to any one of claims 1 to 26, as a method to evaluate the effect, on a T-cell response, of one or more cytokines secreted by a composition of APCs.

Claim 32

The use of a method according to claim 31, wherein the co-incubation of an initial population of T lymphocytes with a composition of antigen-presenting cells (APCs) takes place in the presence of an antibody able to bind specifically to a cytokine that is produced during co-incubation.

Claim 33

The use of a method according to claim 32, wherein the cytokine is chosen among the group consisting of: IL-2, IL-10, IL-12, IL-15, IL-18, IL-23, TNF-α, TGF-β.

Claim 34

Use of a method according to any one of claims 1 to 26, as a method to evaluate the effect, on a T-cell response, of one or more surface determinants markers present on T-cells.

Claim 35

The use of a method according to claim 34, wherein the co-incubation of T-lymphocytes and APCs takes place in the presence of an antibody, or an antagonist, able to bind specifically to a surface determinant marker of T-lymphocytes.

Claim 36

The use of a method according to claim 35, wherein the surface determinant marker is CD4, CD8, CD28, CTLA-4, B7, LFA-10, OX40-ligand, ICAM-1, 4-1BBL, DC-SIGN or MHC-II.

Claim 37

Use of the method according to anyone of claims 1 to 26 as a batch release assay of a composition of APCs.

Claim 38

The use of the method according to claim 37, wherein APCs are characterized by the different percentages of T lymphocytes secreting a cytokine during the co-incubation.

Claim 39

Use of the method according to anyone of claims 1 to 26, as an inclusion criteria for a patient wherein the composition of APCs originating from said patient have the ability to induce a proliferation of T lymphocytes, resulting in a proliferation index at least greater than 2, more preferably of at least 5, more preferably of at least 10, more preferably of at least 15, more preferably of at least 20, more preferably of at least 30, more preferably of at least 50.

Claim 40

Use of the method according to anyone of claims 4 to 26, as an antigen selecting assay wherein the antigen to be tested is loaded on APCs and the T lymphocyte response triggered by the co-incubation with said loaded APCs is compared to the T lymphocyte response induced by a composition of APCs loaded with a reference antigen.

Claim 41

The use according to claim 40, wherein the reference antigen is chosen among the group consisting of: tetanus toxin, Melan-A, Flu peptide, PSA, and HIV.

Claim 42

Use of the method according to anyone of claims 1 to 3 and 5 to 26, to define a standard control T-cell response of T-lymphocytes comprising:

- the co-incubation of an initial population of T-lymphocytes with different compositions of APCs presenting different concentrations of an antigen or of an antigen fragment of interest or of a reference antigen or of a fragment of reference antigen and,
  - the determination of the variation of the degree of proliferation of said T-lymphocytes measured for each composition of APCs according the quantity of said antigen or said fragment of antigen of interest or said reference antigen or said fragment of reference antigen.

Claim 43

The use of the method according to claim 42, to evaluate the efficiency of a process for loading an antigen, or a fragment of antigen, into APCs wherein the efficiency of the said process is evaluated by comparing:

- a first response being a T-cell response of a final population of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with a composition of APCs loaded with an antigen or a fragment of antigen, according to the process to be tested and,
- a second response being a standard control T-cell response of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with different compositions of APCs loaded with different concentrations of said antigen or said fragment of antigen, or of a reference antigen, or of a fragment of reference antigen according to the process of reference,

deducing from said comparison between said first and said second responses the difference of efficiency between the process to be tested and the process of reference.

Claim 44

The use according to claim 43, wherein the reference process is chosen among the group consisting of: fusion, electroporation, incubation, loading with liposomes, loading with virosomes, loading with exosomes or genetic engineering of antigen-presenting cells.

Claim 45

Claim 46

The use of the method according to claim 42, to evaluate an impact of a method of an antigen preparation on the ability of an antigen-presenting cell to present antigen to T lymphocyte wherein the said method of preparation of antigen is evaluated by comparing:

- a first response being a T-cell response of a final population of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with a composition of APCs loaded with an antigen or a fragment of antigen, prepared according to the method to be tested,
- a second response being a standard control T-cell response of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with different compositions of APCs loaded with different concentrations of said antigen or said fragment of antigen, or of a reference antigen, or of a fragment of reference antigen, prepared according to a method of reference,

deducing from said first and said second responses the impact of said method of antigen preparation to be tested on the ability of an antigen-presenting cell to present antigen to T lymphocyte.

Claim 47

The use of the method according to claim 42 to evaluate stability of a presentation of an antigen (or fragment of antigen) by APCs wherein the said stability is evaluated by comparing:

- a first response being a T-cell response of a final population of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with different compositions of APCs loaded with said antigen (or fragment of antigen) said compositions of APCs being previously incubated in a medium not initially containing said antigen for different period of time and,
- a second response being a standard control T-cell response of T-lymphocytes resulting from the co-incubation of an initial population of T-lymphocytes with composition of APCs loaded with an antigen or a fragment of antigen, or a reference antigen, or a fragment of reference antigen said compositions of APCs being not previously incubated in a medium not initially containing said antigen or said fragment of antigen, or a reference antigen, or a fragment of reference antigen,

deducing from the first and the second responses the stability of a presentation of said antigen (or fragment of antigen) by APCs.

Claim 48

The use of the method according to anyone of claims 42 to 47, wherein the initial population of T lymphocytes is a clonal population or a cell line of T lymphocytes that is specific to the antigen, or fragment of antigen, presented in the context of MHC.

Claim 49

The use of the method according to anyone of claims 42 to 47, wherein the initial population of T lymphocytes is an initial naïve population of T lymphocytes, said initial naïve population of T lymphocytes being substantially the same for obtaining a standard control T-cell response of T-lymphocytes and a T-cell response to be compared to said standard control T-cell response of T-lymphocytes.